

AMENDMENTS TO THE CLAIMS

20 1. (Original) A method for identifying a protein with the use of mass
spectrometry, characterized in that
the method is a method in which by referring to sequence information
about a nucleotide sequence of a genomic gene encoding a full-length amino
acid sequence of a peptide chain constituting the known protein, about a
25 nucleotide sequence of a reading frame in mRNA enabling translation of the
full-length amino acid sequence, and about a (deduced) full-length amino acid
sequence encoded by the nucleotide sequence in regard to known individual
proteins, which information is recorded in a database on known proteins, one
of the known proteins recorded in the database which is assessed to correspond
30 to a target protein to be analyzed is selected for the , based on a mass
spectrometric result actually measured for the target protein to be analyzed,
wherein

(1) the mass spectrometric result actually measured for the target protein
is a result obtained from mass spectrometric analysis comprising at least a set of
35 respective actually measured mass values (Mex) of a plurality of peptide
fragments determined by

subjecting a peptide chain isolated in advance that constitutes the target
protein to be analyzed to reduction treatment capable of cleaving disulfide (S-S)
bond in Cys-Cys bond present therein and to treatment that unfolds folding of
40 the target protein to linearize the peptide chain constituting the target protein,

further carrying out treatment for site-specific proteolysis that selectively
cleaves a peptide chain at a particular amino acid or amino acid sequence to
evenly and selectively prepare a plurality of peptide fragments derived from
the linearized peptide chain collected from the target protein, and

45 determining the respective actually measured mass values (M_{ex}) of the
plurality of peptide fragments, based on a result for masses (M) of the plurality
of the peptide fragments produced that is measured by mass spectrometry as
molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation
species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent
50 "parent anion species";

(2) in regard to known individual proteins recorded in said database on
known proteins, referring to sequence information about a nucleotide sequence
of a genomic gene encoding a full-length amino acid sequence of a peptide
chain constituting the known protein, about a nucleotide sequence of a reading
55 frame in mRNA enabling translation of the full-length amino acid sequence,
and about a (deduced) full-length amino acid sequence encoded by the
nucleotide sequence,

calculating predicted molecular weights (M_{ref}) of a plurality of peptide
fragments derived from a peptide chain having said full-length amino acid
60 sequence, presumably produced by subjecting the peptide chain having the
full-length amino acid sequence that is translated according to the genomic
gene encoding the known protein to the reduction treatment for a sulfanyl (-
SH) group on a Cys side chain and to the treatment of site-specific proteolysis
to create a set of the predicted molecular weights (M_{ref}) of the plurality of
65 predicted peptide fragments derived from the known protein, and

employing as a reference standard database, a data set of the predicted
molecular weights (M_{ref}) of the plurality of peptide fragments, wherein the
data set is composed of total sets of the predicted molecular weights (M_{ref}) of
the plurality of known protein-derived predicted peptide fragments calculated
70 for all the known individual proteins recorded in the database on known
proteins;

(3) performing a first comparison operation whereby the set of the respective actually measured mass values (Mex) of the plurality of peptide fragments determined for the target protein to be analyzed is compared with
75 each of the sets of the predicted molecular weights (Mref) of the plurality of known protein-derived predicted peptide fragments calculated for the known individual proteins recorded in the database on known proteins, and
the number of the actually measured peptide fragments derived from the target protein to be analyzed and the number of the known protein-derived
80 predicted peptide fragments judged as having a substantial match between the respective actually measured mass values (Mex) and the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in each of the sets derived from the known proteins in consideration of a measurement error attributed to the utilized mass spectrometry itself are determined each
85 individually for the known proteins comprised in the reference standard database, and
selecting from among the known proteins determined in the first comparison operation, known proteins in decreasing order of the number of the actually measured peptide fragments derived from the target protein to be
90 analyzed and the number of the known protein-derived predicted peptide fragments judged as having a match to classify a known protein exhibiting the highest number of the match into a group of first candidate known protein(s) as a candidate of identification for the target protein to be analyzed; and
(4) when the group of the first candidate known protein(s) comprises one
95 type of known protein, judging the one type of known protein selected from the database as being a single candidate of identification for the target protein to be analyzed.

2. (Original) The method according to claim 1, characterized in that

in the case where in referring to sequence information about the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed,

5 the number of actually measured peptide fragments that are derived from the target protein to be analyzed, which are not judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of
10 identification, is zero,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

3. (Original) The method according to claim 1, characterized in that
in the case where in referring to sequence information about the selected known protein judged in the step (4) as being a single candidate of
identification for the target protein to be analyzed,

5 when arranging the plurality of the actually measured peptide fragments derived from the target protein to be analyzed that are judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of
10 identification, in positions to be occupied by the corresponding predicted peptide fragments derived from the known protein, a group of the actually measured peptide fragments that are judged as having a match constitutes consecutive amino acid sequences that is contained in the full-length amino acid sequence of the known protein,

15 the selected known protein judged in the step (4) as being a single
candidate of identification for the target protein to be analyzed is judged as
being a highly accurate single candidate of identification.

4. (Original) The method according to claim 3, characterized in that
 in the case where there remains a unidentified actually measured
peptide fragment derived from the target protein to be analyzed that is not
judged in the first comparison operation of the step (3) as having a match to the
5 predicted molecular weights (Mref) of the plurality of predicted peptide
fragments in the set derived from the known protein judged as being a
candidate of identification, the method further comprises: in regard to the
unidentified actually measured peptide fragment derived from the target
protein to be analyzed,

10 on the assumption that for a group of predicted peptide fragments which
are linked to the consecutive amino acid sequence portions contained in the
full-length amino acid sequence of the known protein, which are derived from
the known protein judged as being a candidate of identification, and which are
unidentified by the corresponding actually measured peptide fragments, there
15 would exist post-translational modification attributed to modifying group
addition to a side chain of an amino acid residue present in the unidentified
predicted peptide fragments, calculating predicted molecular weights (Mref) of
predicted peptide fragments having the post-translational modification
attributed to modifying group addition to a side chain of an amino acid residue;
20 and

 performing a second comparison operation whereby the presence or
absence of the unidentified actually measured peptide fragment having the
actually measured mass value (Mex) matching to any of the predicted
molecular weights (Mref) of the predicted peptide fragments having the post-

25 translational modification attributed to modifying group addition is judged,
wherein

when at least one unidentified actually measured peptide fragment
derived from the target protein to be analyzed having the actually measured
mass value (Mex) matching to any of the predicted molecular weights (Mref) of
30 the predicted peptide fragments having the post-translational modification
attributed to modifying group addition is selected,

the selected known protein judged in the step (4) as being a single
candidate of identification for the target protein to be analyzed is judged as
being a highly accurate single candidate of identification.

5. (Original) The method according to claim 3, characterized in that
in the case where there remains a unidentified actually measured
peptide fragment derived from the target protein to be analyzed that is not
judged in the first comparison operation of the step (3) as having a match to the
5 predicted molecular weights (Mref) of the plurality of predicted peptide
fragments in the set derived from the known protein judged as being a
candidate of identification, the method further comprises: in regard to the
unidentified actually measured peptide fragment derived from the target
protein to be analyzed,

10 on the assumption that for an N-terminal portion of a group of predicted
peptide fragments which are linked to the consecutive amino acid sequence
portions contained in the full-length amino acid sequence of the known protein,
which are derived from the known protein judged as being a candidate of
identification, and which are unidentified by the corresponding actually
15 measured peptide fragments, post-translational processing of N-terminal
truncation would occur to convert the known protein to a mature protein,
calculating predicted molecular weights (Mref) of a plurality of predicted

peptide fragments derived from the post-translational N-terminal processing, presumably generated by subjecting an assumed amino acid sequence of the known protein to the introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the post-translational N-terminal processing is judged, wherein

when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the post-translational N-terminal processing is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

6. (Original) The method according to claim 3, characterized in that in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, the method further comprises: in regard to the unidentified actually measured peptide fragment derived from the target protein to be analyzed,

10 on the assumption that for a C-terminal portion of a group of predicted
peptide fragments which are linked to the consecutive amino acid sequence
portions contained in the full-length amino acid sequence of the known protein,
which are derived from the known protein judged as being a candidate of
identification, and which are unidentified by the corresponding actually
15 measured peptide fragments, post-translational processing of C-terminal
truncation would occur to convert the known protein to a C-terminally
truncated protein, calculating predicted molecular weights (Mref) of a plurality
of predicted peptide fragments derived from the post-translational processing
of C-terminal truncation, presumably generated by subjecting an assumed
20 amino acid sequence of the known protein to the introduction treatment of a
protecting group and to the site-specific proteolytic treatment; and
 performing a second comparison operation whereby the presence or
absence of the unidentified actually measured peptide fragment derived from
the target protein to be analyzed having the actually measured mass value
25 (Mex) matching to any of the predicted molecular weights (Mref) of the
predicted peptide fragments derived from the post-translational processing of
C-terminal truncation is judged, wherein
 when at least one unidentified actually measured peptide fragment
derived from the target protein to be analyzed having the actually measured
30 mass value (Mex) matching to any of the predicted molecular weights (Mref) of
the predicted peptide fragments derived from the post-translational C-terminal
processing is selected,
 the selected known protein judged in the step (4) as being a single
candidate of identification for the target protein to be analyzed is judged as
35 being a highly accurate single candidate of identification.

7. (Original) The method according to claim 3, characterized in that
in the case where there remains a unidentified actually measured peptide
fragment derived from the target protein to be analyzed that is not judged in
5 the first comparison operation of the step (3) as having a match to the predicted
molecular weights (Mref) of the plurality of predicted peptide fragments in the
set derived from the known protein judged as being a candidate of
identification, the method further comprises: in regard to the unidentified
actually measured peptide fragment derived from the target protein to be
10 analyzed,
on the assumption that in genomic gene portions encoding portions of a
group of predicted peptide fragments which are linked to the consecutive
amino acid sequence portions contained in the full-length amino acid sequence
of the known protein, which are derived from the known protein judged as
15 being a candidate of identification, and which are unidentified by the
corresponding actually measured peptide fragments, splicing different from
presumable RNA splicing in a plurality of exons contained in the genomic gene
portions would occur, calculating predicted molecular weights (Mref) of a
plurality of predicted peptide fragments derived from the alternative splicing,
20 presumably generated by subjecting an assumed amino acid sequence of the
known protein to the introduction treatment of a protecting group and to the
site-specific proteolytic treatment; and
performing a second comparison operation whereby the presence or
absence of the unidentified actually measured peptide fragment derived from
25 the target protein to be analyzed having the actually measured mass value
(Mex) matching to any of the predicted molecular weights (Mref) of the
predicted peptide fragments derived from the alternative splicing is judged,
wherein

when at least one unidentified actually measured peptide fragment
30 derived from the target protein to be analyzed having the actually measured
mass value (Mex) matching to any of the predicted molecular weights (Mref) of
the predicted peptide fragments derived from the alternative splicing is
selected,

the selected known protein judged in the step (4) as being a single
35 candidate of identification for the target protein to be analyzed is judged as
being a highly accurate single candidate of identification.

8. (Original) The method according to claim 3, characterized in that
in the case where there remains a unidentified actually measured peptide
fragment derived from the target protein to be analyzed that is not judged in
the first comparison operation of the step (3) as having a match to the predicted
5 molecular weights (Mref) of the plurality of predicted peptide fragments in the
set derived from the known protein judged as being a candidate of
identification, the method further comprises: in regard to the unidentified
actually measured peptide fragment derived from the target protein to be
analyzed,

10 on the assumption that in portions of a group of predicted peptide
fragments which are linked to the consecutive amino acid sequence portions
contained in the full-length amino acid sequence of the known protein, which
are derived from the known protein judged as being a candidate of
identification, and which are unidentified by the corresponding actually
15 measured peptide fragments, protein splicing that removes a portion of an
amino acid sequence thereof would occur, calculating predicted molecular
weights (Mref) of a plurality of predicted peptide fragments derived from the
protein splicing, presumably generated by subjecting an assumed amino acid

sequence of the known protein to the introduction treatment of a protecting
20 group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or
absence of the unidentified actually measured peptide fragment derived from
the target protein to be analyzed having the actually measured mass value
(Mex) matching to any of the predicted molecular weights (Mref) of the
25 predicted peptide fragments derived from the protein splicing is judged,
wherein

when at least one unidentified actually measured peptide fragment
derived from the target protein to be analyzed having the actually measured
mass value (Mex) matching to any of the predicted molecular weights (Mref) of
30 the predicted peptide fragments derived from the protein splicing is selected,

the selected known protein judged in the step (4) as being a single
candidate of identification for the target protein to be analyzed is judged as
being a highly accurate single candidate of identification.

9. (Original) The method according to claim 3, characterized in that
in the case where there remains a unidentified actually measured peptide
fragment derived from the target protein to be analyzed that is not judged in
the first comparison operation of the step (3) as having a match to the predicted
5 molecular weights (Mref) of the plurality of predicted peptide fragments in the
set derived from the known protein judged as being a candidate of
identification, the method further comprises: in regard to the unidentified
actually measured peptide fragment derived from the target protein to be
analyzed,

10 on the assumption that for genomic gene portions encoding a group of
predicted peptide fragments which are linked to the consecutive amino acid
sequence portions contained in the full-length amino acid sequence of the

known protein, which are derived from the known protein judged as being a candidate of identification, and which are unidentified by the corresponding actually measured peptide fragments, one replacement of a translated amino acid attributed to single nucleotide polymorphism would occur in an exon contained in the genomic gene portions, calculating predicted molecular weights (Mref) of a plurality of predicted peptide fragments derived from the amino acid replacement of single nucleotide polymorphism, presumably generated by subjecting an assumed amino acid sequence of the known protein to the introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the amino acid replacement of single nucleotide polymorphism is judged, wherein

when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the amino acid replacement of single nucleotide polymorphism is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

10. (Original) The method according to claim 1, characterized in that

in the case where in referring to sequence information about the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed, and

5 arranging the plurality of the actually measured peptide fragments derived from the target protein to be analyzed that are judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of
10 identification, in positions to be occupied by the corresponding predicted peptide fragments derived from the known protein,

 a group of the actually measured peptide fragments that is judged as having a match constitutes consecutive amino acid sequences contained in the full-length amino acid sequence of the known protein except for positions to be
15 occupied by some predicted peptide fragments,

 the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

11. (Original) The method according to claim 10, characterized in that
 in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted
5 molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, the method further comprises: in regard to the unidentified actually measured peptide fragment derived from the target protein to be analyzed,

10 on the assumption that for a group of predicted peptide fragments which
are located within the consecutive amino acid sequences portions contained in
the full-length amino acid sequence of the known protein, which are derived
from the known protein judged as being a candidate of identification, and
which are unidentified by the corresponding actually measured peptide
15 fragments, there would exist post-translational modification attributed to
modifying group addition to a side chain of an amino acid residue present in
the unidentified predicted peptide fragments, calculating predicted molecular
weights (Mref) of predicted peptide fragments having the post-translational
modification attributed to modifying group addition to a side chain of an amino
20 acid residue; and

 performing a second comparison operation whereby the presence or
absence of the unidentified actually measured peptide fragment derived from
the target protein to be analyzed having the actually measured mass value
(Mex) matching to any of the predicted molecular weights (Mref) of the
25 predicted peptide fragments having the post-translational modification
attributed to modifying group addition is judged, wherein

 when at least one unidentified actually measured peptide fragment
derived from the target protein to be analyzed having the actually measured
mass value (Mex) matching to any of the predicted molecular weights (Mref) of
30 the predicted peptide fragments having the post-translational modification
attributed to modifying group addition is selected,

 the selected known protein judged in the step (4) as being a single
candidate of identification for the target protein to be analyzed is judged as
being a highly accurate single candidate of identification.

12. (Original) The method according to claim 10, characterized in that

in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, the method further comprises: in regard to the unidentified actually measured peptide fragment derived from the target protein to be analyzed,

on the assumption that in genomic gene portions encoding portions of a group of predicted peptide fragments in an internal unidentified region which are located within the consecutive amino acid sequence portions contained in the full-length amino acid sequence of the known protein, which are derived from the known protein judged as being a candidate of identification, and which are unidentified by the corresponding actually measured peptide fragments, splicing different from presumable RNA splicing in a plurality of exons contained in the genomic gene portions would occur, calculating predicted molecular weights (Mref) of a plurality of predicted peptide fragments derived from the alternative splicing, presumably generated by subjecting an assumed amino acid sequence of the known protein to the introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the different splicing is judged, wherein

when at least one unidentified actually measured peptide fragment
30 derived from the target protein to be analyzed having the actually measured
mass value (Mex) matching to any of the predicted molecular weights (Mref) of
the predicted peptide fragments derived from the alternative splicing is
selected,

the selected known protein judged in the step (4) as being a single
35 candidate of identification for the target protein to be analyzed is judged as
being a highly accurate single candidate of identification.

13. (Original) The method according to claim 10, characterized in that
in the case where there remains a unidentified actually measured peptide
fragment derived from the target protein to be analyzed that is not judged in
the first comparison operation of the step (3) as having a match to the predicted
5 molecular weights (Mref) of the plurality of predicted peptide fragments in the
set derived from the known protein judged as being a candidate of
identification, the method further comprises: in regard to the unidentified
actually measured peptide fragment derived from the target protein to be
analyzed,

10 on the assumption that in portions of a group of predicted peptide
fragments in an internal unidentified region which are located within the
consecutive amino acid sequence portions contained in the full-length amino
acid sequence of the known protein, which are derived from the known protein
judged as being a candidate of identification, and which are unidentified by the
15 corresponding actually measured peptide fragments, protein splicing that
removes a portion of an amino acid sequence thereof would occur, calculating
predicted molecular weights (Mref) of a plurality of predicted peptide
fragments derived from the protein splicing, presumably generated by
subjecting an assumed amino acid sequence of the known protein to the

20 introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value
25 (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the protein splicing is judged, wherein

when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured
30 mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the protein splicing is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

14. (Original) The method according to claim 10, characterized in that

in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted
5 molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, the method further comprises: in regard to the unidentified actually measured peptide fragment derived from the target protein to be analyzed,

10 on the assumption that for genomic gene portions encoding respective portions of a group of predicted peptide fragments in an internal unidentified region which are located within the consecutive amino acid sequence portions

contained in the full-length amino acid sequence of the known protein, which are derived from the known protein judged as being a candidate of
15 identification, and which are unidentified by the corresponding actually measured peptide fragments, one substitution of a translated amino acid attributed to single nucleotide polymorphism would occur in an exon contained in the genomic gene portions, calculating predicted molecular weights (Mref) of a plurality of predicted peptide fragments derived from the amino acid
20 substitution of single nucleotide polymorphism, presumably generated by subjecting an assumed amino acid sequence of the known protein to the introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or
25 absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the amino acid substitution of single nucleotide polymorphism is judged, wherein

30 when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the amino acid substitution of single nucleotide polymorphism is selected,

35 the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

15. (Currently Amended) The method according to ~~any one of claims 4 to 9 and 11 to 14~~, claim 4, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular

weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

16. (Currently Amended) The method according to ~~any one of claims 1 to 15~~, claim 1, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

17. (New) The method according to claim 5, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M)

of the plurality of generated peptide fragments measured by mass spectrometry as molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

18. (New) The method according to claim 6, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be

analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

19. (New) The method according to claim 7, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as

molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

20. (New) The method according to claim 8, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be

analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

21. (New) The method according to claim 9, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as

molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

22. (New) The method according to claim 11, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be

analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

23. (New) The method according to claim 12, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (Mex) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as

molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

24. (New) The method according to claim 13, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be

analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

25. (New) The method according to claim 14, characterized in that

the method further comprises: at least in the second comparison operation, utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (M_{ex}) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by mass spectrometry as

molecular weights ($M+H/Z$; $Z=1$) of corresponding monovalent "parent cation species" or as molecular weights ($M-H/Z$; $Z=1$) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (M_{ex}) matching to any of the predicted molecular weights (M_{ref}) of the predicted peptide fragments,

performing comparison whereby molecular weights of fragmented derivative ion species presumably generated in MS/MS analysis due to the assumed amino acid sequence and additional modification group constituting the corresponding predicted peptide fragment are also compared with the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed; and

when correspondence relationship is also confirmed at least between the actually measured result of the molecular weights of the fragmented derivative ion species for the actually measured peptide fragment derived from the target protein to be analyzed and the predicted values of the molecular weights of the predicted fragmented derivative ion species for the corresponding predicted peptide fragment,

regarding as judgment with high accuracy, the judgment of the actually measured peptide fragment derived from the target protein to be analyzed selected in the second comparison operation, wherein

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

26. (New) The method according to claim 2, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

27. (New) The method according to claim 3, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

28. (New) The method according to claim 4, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

29. (New) The method according to claim 5, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

30. (New) The method according to claim 6, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

31. (New) The method according to claim 7, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

32. (New) The method according to claim 8, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

33. (New) The method according to claim 9, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group

for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

34. (New) The method according to claim 10, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

35. (New) The method according to claim 11, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

36. (New) The method according to claim 12, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

37. (New) The method according to claim 13, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

38. (New) The method according to claim 14, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.

39. (New) The method according to claim 15, characterized in that

the method further comprises prior to the site-specific proteolytic treatment, performing on the linearized peptide chain, selective introduction of a protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare the resulting linearized peptide chain having the protected Cys.